Effect of Temperature on Ultrafast Separation of Macromolecules

Application

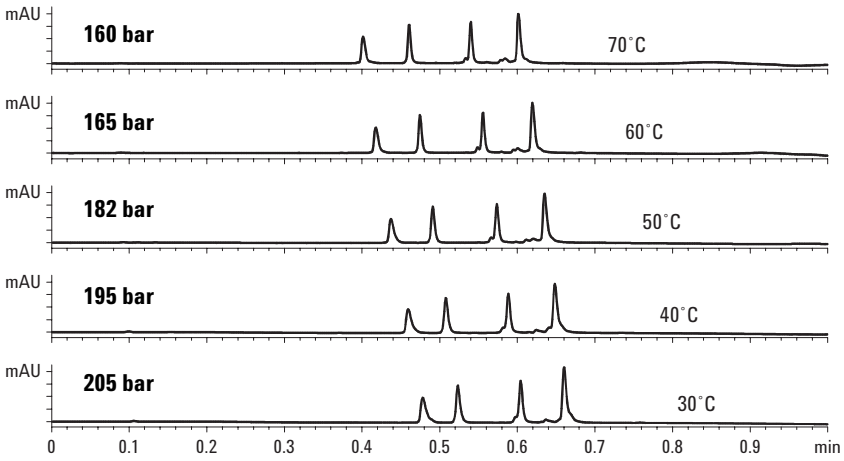
Technical

Robert D. Ricker and Cliff Woodward*

In the modern protein laboratory, sensitivity, speed, and resolution are sometimes competing goals. Frequently the analyst is working under conditions of limited sample size, a large number of samples, and a need for resolution of contaminants in the shortest possible time. Under these circumstances, Poroshell 300SB shines, delivering ultrasharp peaks with ultrashort run times and less required sample (20% that of a 4.6-mm id column). As may be seen in the chromatograms below, increased temperature enables shorter run times, increased resolution by sharpened peaks, and reduced mobile-phase viscosity for maximal throughput as a result of higher flow rates.

Poroshell 300SB-C18

2.1 × 75 mm, 5 µm; part no: 660750-902



Conditions:

Agilent 1100 HPLC; WPS with delay-volume reduction

Thermostatted column compartment

Mobile phase:

A = 95% H_2O, 5% ACN with 0.1% TFA, pH 2.1

B = 5% H_2O, 95% ACN with 0.07% TFA

Flow = 2 mL/min

Piston stroke: 20 µL

UV: 215 nm

Temperature: 70°C

Compound analyzed (1 µL/injection, approximate 250 µg/mL):

1. Neurotensin
2. RNase A
3. Lysozyme
4. Myoglobin

Highlights

- Poroshell 300SB-C18 uses proven StableBond technology to maintain stability at elevated temperatures and low pH.
- Because of the thin superficially porous layer, Poroshell 300SB-C18 allows rapid equilibration with large molecules having slower diffusion into pores.
- Use of higher temperatures reduces system back pressure and mobile-phase viscosity enabling higher flow rates to be used.
- Poroshell 300SB-C18 allows the use of higher linear velocities, with no loss in resolution. This translates into shorter run times.
- Poroshell columns were designed with a 2.1-mm id so that high linear velocities could be achieved at typical flow rates; 2 mL/min on this column compares to nearly 10 mL/min on a conventional 4.6-mm id column.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

*Robert Ricker and Cliff Woodward are application chemists based at Agilent Technologies, Wilmington, Delaware.

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2002

Printed in the USA
April 10, 2002
5988-5673EN

Agilent Technologies